SAFETY AND EFFICACY OF A HEPATITIS E VIRUS VACCINE CONDUCTED IN NEPAL

Shrestha M.P., Scott R.M., and Shrestha S.K.
Walter Reed/AFRIMS Research Unit Nepal (WARUN)
Kathmandu, Nepal

Myint K.S.A.*, and Mammen M.P.
Department of Virology, Armed Forces Institute of Medical Sciences (AFRIMS)
Bangkok, Thailand

Joshi D.M., Thapa G.B., and Thapa N.
Nepalese Army
Kathmandu, Nepal

Fourneau M., David M.P., Safary A., and Innis B.L.
GlaxoSmithKline Biologicals
Rixensart, Belgium

Kuschner R.A., Seriwatana J., and Endy T.P.
Division of Communicable Diseases and Immunology, Walter Reed Army Institute of Research (WRAIR)
Silver Spring, MD

Vaughn D.W.
Military Infectious Diseases Research Program, US Army Medical Research and Materiel Command
Fort Detrick, MD

ABSTRACT

Hepatitis E virus (HEV) causes hepatitis E, a public health problem in many developing countries. Several outbreaks of hepatitis E have been reported in military environments. We evaluated the safety and efficacy of a HEV recombinant protein (rHEV) vaccine in a randomized, double-blinded, placebo-controlled trial.

In Nepal, we studied 2,000 healthy adults susceptible to HEV who were randomized to receive 3 doses of either the rHEV vaccine (1,000) or placebo (1,000) at months 0, 1 and 6. Active and hospital surveillance were used to identify acute hepatitis and adverse events. The primary endpoint was hepatitis E occurring after 3 vaccine doses.

1,794 volunteers (896 in the vaccine group, 898 in the placebo group) received 3 vaccine doses; the total vaccinated cohort was followed a median of 804 days. 66 of 69 hepatitis E cases after dose 3 occurred in placebo recipients; vaccine efficacy was 95.5 percent (95 percent confidence interval 85.6 to 98.6 percent). An intention to treat analysis considering all 87 hepatitis E cases occurring after dose 1 found 9 cases among vaccine recipients and estimated vaccine efficacy to be 88.5 percent (95 percent confidence interval 77.1 to 94.2 percent). The proportions of subjects reporting solicited general symptoms over 8 days or unsolicited symptoms over 31 days after any vaccine dose were similar between groups. In the total vaccinated cohort, there were fewer serious adverse events in the vaccine group compared to placebo ($p<0.001$).

In a high risk population, this rHEV vaccine was well tolerated, safe and efficacious in preventing hepatitis E. Additional studies might be needed to establish the vaccine's safety and immunogenicity in other populations for subsequent licensure of the Hepatitis E vaccine to protect soldiers and travelers in endemic areas.

1. INTRODUCTION

Hepatitis E virus (HEV) is the most frequent cause of viral hepatitis for which there is no vaccine; hepatitis E is a major public health problem in many developing countries (Labrique et al., 1999). The disease occurs sporadically and in epidemics, causing considerable morbidity and mortality, especially in pregnant women (Worm et al., 2002). Based on seroprevalence, an estimated one third of the world's population has been infected with HEV (Purcell and Emerson, 2005). In India, the lifetime infection risk surpasses 60 percent, which translates to hundreds of thousands of illnesses annually (Worm and Wirnsberger, 2004). Hepatitis E is self-limited and occurs typically where access to care is restricted and laboratory diagnosis is unavailable

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01 NOV 2006	**N/A**	**-**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Safety And Efficacy Of A Hepatitis E Virus Vaccine Conducted In Nepal	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Walter Reed/AFRIMS Research Unit Nepal (WARUN) Kathmandu, Nepal**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES
See also ADM002075.

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**UU**	**5**	

(Krawczynski et al., 2005). Consequently, the true hepatitis E disease burden is unknown.

In hepatitis E outbreaks, the average incubation period is approximately 40 days; the highest attack rates are in those 15 to 40 years old (Emerson and Purcell, 2003). Illness severity increases with age; the overall case-fatality ratio is 1 to 3 percent (Purcell, 1994). Pregnant women have the highest risk for associated acute hepatic failure; their case fatality ratio is 5 to 25 percent; survivors have high rates of spontaneous abortion and stillbirth (Krawczynski et al., 2005). Several outbreaks of hepatitis E have been reported in military environments in Chad, Djibouti, Nepal, Ethiopia, and among Bangladeshi soldiers serving with the United Nations (UN) Forces in Haiti. French and Italian soldiers serving in Somalia have been reported to be infected with HEV. Outbreaks also occur in military and paramilitary forces in India, as well as in military units in Sargodha and Abbottabad in Pakistan. High attack rates and lengthy convalescent periods contribute significantly to loss of soldier duty days and seriously impact military operations.

HEV, a non-enveloped, single-stranded, positive sense RNA virus, genus Hepevirus, has a genome comprised of three overlapping open reading frames (ORFs); ORF 2 encodes the principal capsid protein. There are four HEV genotypes: genotype 1 causes most human disease; genotype 2 is rare; genotypes 3 and 4, while prevalent in domestic animals like swine, may have reduced pathogenicity for humans (Emerson and Purcell, 2003). Nevertheless, all HEV can be considered to belong to one serotype (Worm, 2004). Therefore, a vaccine shown to be efficacious in one country could protect against hepatitis E anywhere, as long as it elicited similar immune responses.

A genotype 1 HEV recombinant protein (rHEV) vaccine protected non-human primates (Tsarev et al., 1997). In American adults, intramuscular administration of rHEV vaccine on a 0, 1 and 6-month schedule was well tolerated and immunogenic over a range of doses (5 to 40 μg) (Safary, 2001). Likewise, in Nepalese adults, the vaccine was well tolerated and immunogenic (personal communication). These results formed the basis for a clinical trial to establish the vaccine's efficacy in volunteers from the Nepalese Army, a population at high risk for hepatitis E (Clayson et al., 1997, 1998).

2. METHODS

2.1 Research Oversight and Informed Consent

Institutional review boards of the Nepal Health Research Council and the United States Army approved the study protocol. The United States Army Medical Materiel Development Activity monitored trial conduct and data veracity. An independent Data and Safety Monitoring Board (DSMB) monitored adverse events and confirmed endpoints prior to unblinding. Each volunteer gave written consent prior to participation.

2.2 Study Design

5,323 healthy male and non-pregnant female volunteers, age 18 years and older, were recruited from 61 Nepalese Army units in Kathmandu. Serology was used to assess eligibility (Innis et al., 2002); 66.3 percent were eligible with anti-rHEV imunoglobulin (Ig) <20 Walter Reed (WR) U/mL. Of these, volunteers with anti-rHEV Ig <10 WR U/mL were randomized initially (N=1,885); 115 with anti-rHEV Ig ≥10 WR U/mL to <20 WR U/mL were randomized subsequently to reach a total of 2,000 from 45 Army units. All volunteers were randomized at one site, without stratification. During the double-blinded trial, no one was unblinded.

2.3 Vaccine and Placebo

The vaccine was a purified polypeptide produced in Spodoptera frugiperda cells infected with a recombinant baculovirus containing a truncated HEV genomic sequence encoding the capsid antigen (Robinson et al., 1998). A vaccine dose contained 20 μg rHEV antigen in 0.5 mL adsorbed to 0.5 mg aluminium hydroxide. An identically-appearing placebo dose contained 0.5 mg aluminum hydroxide in 0.5 mL saline. Three doses of vaccine or placebo were administered intramuscularly on a 0, 1 and 6-month schedule.

2.4 Case Definition and Evaluation of Efficacy and Immunogenicity

Hepatitis cases were identified through active bi-weekly surveillance at military units and daily hospital surveillance. Definite hepatitis E was defined as jaundice or illness for at least three days, with at least three of the following: fatigue, appetite loss, abdominal discomfort, right upper quadrant abdominal pain, nausea, vomiting (Clayson et al., 1995); liver injury had to be confirmed by serum alanine aminotransferase greater than 2.5 times the normal upper limit or serum total bilirubin greater than 2 mg/dL (34 μmol/L); HEV RNA had to be detected in serum or stool by RT-PCR (Tsarev et al., 1999); HEV infection had to be confirmed by detection of either anti-rHEV IgM ≥100 WR U/mL (Seriwatana et al., 2002) or anti-rHEV Ig ≥2500 WR U/mL. Probable hepatitis E was defined similarly, but allowed case confirmation using a lower anti-rHEV Ig criterion of 1,000 WR U/ml. The immune response at months 0, 2, 6, 7 and 24 was determined by anti-rHEV Ig immunoassay using the vaccine antigen (Innis et al., 2002).

2.5 Assessment of Safety

Adverse events were solicited at study visits; additionally, all clinic visits and hospital admissions were reviewed daily to identify volunteers. A randomly selected reactogenicity subset was interviewed on days 1, 3, 5 and 7 after each vaccination to record injection site findings and general symptoms. Serious adverse events (medically significant or resulting in hospitalization, disability or death) were recorded throughout the study.

2.6 Study Endpoints and Statistical Analysis

The primary efficacy endpoint was prevention of definite hepatitis E occurring at least 14 days after vaccine dose 3. A secondary efficacy endpoint was prevention of definite hepatitis E occurring from 14 days after dose 2 until dose 3.

We estimated the hepatitis E attack rate in placebos would be 1.6 percent over one year (Clayson et al., 1997, 1998). Assuming a true vaccine efficacy of 80 percent, a two-group continuity corrected χ^2 test with a 0.05 one-sided significance level would have 80 percent power to detect a difference in the incidence of hepatitis E with 866 per group (Nquery Advisor, Version 5.0). To compensate for dropouts, a sample size of 1,000 per group was planned.

The vaccine efficacy cohort included all who received 3 doses for the primary analysis and all who received 2 doses for a secondary analysis. A two-sided Fisher's exact test was used to compare the percentage with hepatitis E between groups. A two-sided 95 percent confidence interval for vaccine efficacy (1-relative risk) was computed (Mantel-Haenszel confidence interval for relative risk).

For robustness, efficacy also was computed in the total vaccinated cohort (those who received at least one vaccine dose) based on the relative risk and by the Cox regression model; cumulative incidence as hazard ratio curves, including the group effect as regressor, was generated to analyze time to occurrence of hepatitis E. The log rank test was used to compare the groups.

In a reactogenicity subset (N=200), the proportion reporting solicited symptoms during 8 days after vaccination was compared between groups. In the total vaccinated cohort minus the reactogenicity subset (N=1,800) and in the reactogenicity subset, the proportions reporting unsolicited adverse events during 31 days after any dose were compared between groups. In the total vaccinated cohort, the occurrence of serious adverse events (SAEs) was compared between groups. All comparisons used a two-sided Fisher's exact test.

In a randomly selected immunogenicity subset of those who complied with all protocol vaccination and blood sampling requirements (N=80 vaccine and 160 placebo), the proportion with anti-rHEV Ig ≥20 WR U/mL and the geometric mean concentrations (GMC) of anti-rHEV Ig were analyzed.

Data analysis was performed using SAS software (version 8.2) and ProcStatXact 5 with Windows NT 4.0. All p-values are 2-sided.

3. RESULTS

The detailed results of this study have been submitted to an international peer-review journal for potential publication. We provide here a brief synopsis of the results.

3.1 Study Population

After screening, 2,000 healthy volunteers (99.6% male), age 25.2 ± 6.25 years (mean ± SD) (range 18-62 years), were randomized July to August 2001. Follow-up ended January 2004. Groups (1,000 each) were comparable with respect to mean age (vaccine, 25.2 years; placebo, 25.1 years) and sex (4 females per group). Withdrawals were similar between groups; 1,566 were followed a median of 804 days.

3.2 Vaccine Efficacy

The DSMB reviewed 111 hepatitis episodes and certified 87 hepatitis E cases. Of these, all were definite; none was probable; 84 were icteric and 3 were anicteric (all placebo). The primary objective was to evaluate efficacy of a 3-dose vaccination course. From 14 days after dose 3, there were 69 cases: 3 vaccine (attack rate 0.3 percent) and 66 placebo (attack rate 7.4 percent) ($P<0.001$; Fisher's exact test); vaccine efficacy was 95.5 percent (95 percent confidence interval, 85.6 to 98.6 percent).

3.3 Safety

Similar loss to follow-up (21.8 percent vaccine, 20.7 percent placebo) suggested vaccine was as well tolerated as placebo. Solicited symptom reporting rates were similar between groups except for injection site pain. There were no allergic reactions or anaphylaxis.

The proportion of subjects reporting any unsolicited adverse event was similar between groups. Likewise, the proportion reporting any unsolicited adverse event which prevented normal activities was similar between groups.

Serious adverse events (SAEs) occurred in 14.3 percent of vaccine recipients and 20.0 percent of placebo recipients (P<0.001; Fisher's exact test). The most common SAE category was infections and infestations (83 of 143 SAEs, vaccine; 154 of 200 SAEs, placebo).

4. DISCUSSION

The rHEV vaccine was well tolerated, safe and highly protective against hepatitis E over a median 804 days of follow-up. The primary analysis estimated 3-dose vaccine efficacy to be 95.5 percent. The intention to treat analysis supports this finding, estimating rHEV vaccine efficacy from dose 1 to be between 88.5 and 89.9 percent.

Vaccination was conducted while HEV was being transmitted in the study population, affording an opportunity to evaluate onset of protection. Before a second dose, 4 vaccine recipients developed hepatitis E (days 1, 13, 13 and 30) compared to one placebo recipient (day 5). The other four hepatitis E cases among 1-dose recipients (1 vaccine, 3 placebo) occurred 104 to 288 days after vaccination; no protection can be concluded after one dose. Vaccination with 2 doses may afford protection, but the study was too small to confirm this finding (vaccine efficacy of 86%, with a 95% CI including zero). These results suggest that, while rHEV vaccine after 2 doses might contribute to control of hepatitis E outbreaks, this requires confirmation.

The rHEV vaccine was safe. The profile of solicited symptoms was no different from placebo, except for injection site pain occurring more frequently among vaccine recipients. There were no differences in unsolicited adverse events after any vaccine dose between groups; SAEs were reduced in vaccine recipients, principally by prevention of hepatitis E, a frequent cause of hospitalization in this population. Although the sample size was too small to exclude rare vaccine-related adverse events, there was no suggestion of any safety concern.

Our study was conducted almost exclusively in males. In 2 earlier rHEV vaccine trials, females had anti-rHEV Ig responses similar to males (unpublished data). In view of the mechanistic and temporal association between anti-rHEV Ig and vaccine protection, similar vaccine immunogenicity between sexes suggests that our efficacy results should apply to females.

In summary, we demonstrated that 3 doses of the rHEV vaccine prevent hepatitis E in adult males living where HEV is endemic. Additional studies will be required to establish the vaccine's safety and immunogenicity in other populations for subsequent licensure of the Hepatitis E vaccine to protect soldiers and travelers in endemic areas.

ACKNOWLEDGEMENTS

The authors are deeply grateful to the volunteers who participated in this clinical study as well as to the Nepalese Army Headquarters and Medical Department, our colleagues at the Armed Forces Research Institute of Medical Sciences (AFRIMS), the Walter Reed/AFRIMS Research Unit Nepal (WARUN), the United States Army Medical Materiel Development Activity (USAMMDA) and the Walter Reed Army Institute of Research (WRAIR). We thank Dr. Robert H. Purcell and Dr. Susanne U. Emerson of the Laboratory of Infectious Diseases, U.S. National Institute of Allergy and Infectious Diseases, and Dr. Charles English and Dr. Eileen Villasante, USAMMDA, for their guidance and support throughout this study. Jacques Bruhwyler assisted in this report's preparation. Additional acknowledgement goes to the United States Embassy Nepal, the Nepal Health Research Council, and the members of the DSMB and its Chairman, Dr. John G. Dickinson.

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